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We salute Herbert Laboratories for their contribution to the Endowment Fund and for their continued support of clinical and investigative dermatology.

D.A.N., Denver, CO

In This Issue . . .

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Keratinocyte Differentiation Is Accompanied By Changes in the Organization of Keratin Intermediate Filaments, Microtubules, and Microfilaments

As a cell embarks down the path of differentiation, the ensuing changes in morphology and function are associated with alterations in the cellular skeleton, which is comprised of three major filamentous structures: intermediate filaments, microtubules, and microfilaments. Understanding the changes in the cytoskeleton that occur with normal differentiation may provide the necessary background for learning how the cytoskeleton may be altered in states of aberrant differentiation, such as in cancer.

The keratinocyte is an ideal cell in which differentiation-associated changes in the cytoskeleton may be studied: it can now be cultured in the laboratory with relative ease, can be maintained as a proliferative cell in a culture medium with a low calcium concentration, and can be stimulated to differentiate by increasing the calcium concentration in the medium. As reported in this issue of the *JID,* Dr. Glenn Zamansky and colleagues at Boston University have employed the calcium-induced differentiation of cultured human keratinocytes as a model for studying the differentiation-associated changes in the cytoskeleton. They believe that the cytoskeletal alterations due to calcium reflect changes that occur in normal cellular differentiation.

Dr. Zamansky's technique for identifying the cytoskeleton involves simultaneous immunofluorescence staining either for the protein tubulin of microtubules and the actin of microfilaments, or for the keratin of intermediate filaments and the actin of microfilaments. In keratinocytes proliferating in low calcium medium, intermediate filaments and microtubules tend to localize together in the central part of the cell near the nucleus with some radiation into the cytoplasm, whereas microfilaments occur as encircling bands of parallel bundles on the periphery of the cell. With calcium-induced differentiation, keratin filaments also appear radiating inward from the cell membrane at sites of cell-to-cell apposition, and microfilaments reorganize to make an encasing ring of concentric bundles at the periphery of the cell, with short actin bundles extending toward the cell membrane, especially at the sites where adjacent cells touch. These structural changes are evident as early as 30 minutes after the switch of cultured cells to high calcium. The non-membrane-associated intermediate filaments and the microtubules appear to co-localize predominantly in the areas enclosed by the microfilament network and are diminished in the areas of dense microfilaments. If microfilaments are disrupted by cytochalasin D, the keratin filaments assume a lattice-like structure, and focal aggregates of both keratin and actin appear within the lattice. The membrane-associated keratin filaments persist despite the disruption of microfilaments and other changes in the intermediate filament network. Disruption of microfilaments leads to a more diffuse distribution of microtubules within the cell. If demecolcine is added to low-calcium keratinocyte cultures in order to depolymerize microtubules, microfilament rings, similar to those seen in high calcium, are formed. The maturation-associated changes in the keratin network appear to be unaltered by disruption of microtubules.

Dr. Zamansky believes that these results indicate that microfilaments are somehow involved in the cytoplasmic distribution of microtubules and intermediate filaments, which are located together within the encirclement of microfilaments, and that the actin of microfilaments, at least focally, is intimately associated with keratin filaments. Because the alignment of keratin at sites of cell-to-cell apposition is not prevented by disruption of microfilaments, although keratin filaments near the cell membrane may be closely associated with actin, it appears that microfilaments are not necessary for the formation of the membrane-associated keratin filaments. Microtubules and keratin filaments do not appear to depend on one another for formation of their cytoskeleton networks. Knowing how the cytoskeletal components change with normal differentiation and how they may interrelate provides an essential background for studying how they are different in states of aberrant differentiation and may provide important clues to the behavior of cancer cells.

The Dowling-Meara Type of Epidermolysis Bullosa Simplex Represents a Defect in Basal Cell Keratins

Investigators pursuing the basic causes of the genetically determined mechanico-bullous diseases, collectively known as epidermolysis bullosa (EB), have had few clues regarding exactly which structures and which proteins in the skin are abnormal. One notable

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exception is an unusual type of EB first described in 1954 by Dowling and Meara. Individuals with this disease present with relatively widespread blisters at or soon after birth. The blisters do not typically lead to scarring and tend to cluster, similar to the blisters of dermatitis herpetiformis. With careful microscopic examination of early blisters, it appears that the blisters form along the base of the epidermis due to lysis of basal cells, thus making this type similar to other types of EB simplex. A most unusual finding noted in the skin biopsies is a "clumping" of the tonofilaments in the basal cells. Logical hypotheses that emerge from these observations are that this type of EB might be due to an abnormality of a keratin that makes up these clumped tonofilaments and that this abnormality in a cytoskeletal protein determines the fragility of the cells and the easy blistering.

In pursuit of these hypotheses, Dr. Robin Eady and colleagues in Britain have carefully examined skin biopsies from a large number of patients with the Dowling-Meara type of EB simplex for the presence of tonofilament clumping, and have characterized the types of keratins in the clumps by immunocytochemistry. Their study, reported in this issue of the *JID*, provides valuable clues to the pathogenesis of this type of EB, and, according to Dr. Eady, helps lay the foundation so that molecular biologists may more precisely define the defect.

Dr. Eady's group first provided evidence that the presence of the tonofilament clumps is an intrinsic abnormality in these patients' skin and not a phenomenon somehow secondary to blister formation. The clumps were found not only in basal cells but also within keratinocytes in the spinous cell layer. Clumps were noted in the epidermis of normal-appearing skin and in epithelial cells of adnexal structures. Even in keratinocytes cultured from patients' skin, the clumped tonofilaments could be found by electron microscopy. Similar morphologic abnormalities were not noted in skin biopsies of other types of EB simplex. When the skin biopsies were studied with antibodies to different types of keratins by immunofluorescence and immunoelectron microscopy, the abnormal clumps of tonofilaments stained strongly for the pair of keratins characteristic of basal cells, K5 and K14.

These observations lead to an even more refined hypothesis: that the Dowling-Meara type of EB simplex is due to an abnormality in the cytoskeleton of keratinocytes, involving the basal cell keratins, K5 or K14. As predicted by Dr. Eady, scientists studying the molecular biology of keratins are now using these clues to define more specifically the defect in this disease. Dr. Elaine Fuchs and colleagues have reported in *Cell* in September 1991 that the defect in this disease is a point mutation in a critical region of the K14 keratin gene and that this mutation leads to faulty assembly of keratin filaments. Perhaps now, with this disease as a model, investigators will be able to define similar defects in the keratinocyte cytoskeleton in the other types of EB simplex.

Advanced Techniques in Molecular Biology: Adaptation for Study of the Skin

Increasingly, molecular biology is becoming an integral part of biologic research. This issue of the *JID* contains three papers that focus on advanced laboratory techniques that may be used to study the molecular biology of the skin. One paper describes a successful method for constructing a full-length DNA complementary to messenger RNA (mRNA) for a collagen protein; one presents three methods for preparing mRNA from the skin; and one analyzes methods for transfecting keratinocytes with exogenous DNA.

A starting point in molecular biology investigation is the synthesis of DNA strands complementary to mRNA. Because mRNA for collagen proteins are large and are folded due to a high content of guanine and cytosine, making full-length cDNA has often been technically difficult. Dr. Charlotte Phillips and colleagues at Duke describe the use of a different strategy to construct a full-length cDNA for murine proα2 (I) collagen. In this technique, reverse transcription is performed in the presence of an oligonucleotide that binds to the polyA tail of mRNA and that has an adapter sequence with rare restriction enzyme sites. Amplification of the desired cDNA is accomplished by the polymerase chain reaction (PCR), with one oligonucleotide primer being the adapter sequence and the other being a known region of the gene. The amplified cDNA can then be isolated, cut with appropriate enzymes, and inserted into a vector. This strategy allows construction of cDNA for a collagen molecule and obviates the need for screening an enormous cDNA library.

Study of mRNA normally expressed in the human epidermis has not been totally satisfactory because of the difficulty in performing and interpreting in situ hybridization and because of questions whether mRNA from cultured epidermal cells is a valid representation of mRNA expressed in vivo. Dr. Jack Longley at Yale and collaborators describe methods of isolating mRNA from the skin and demonstrate the utility of these mRNA for molecular biology techniques. Relatively large quantities of mRNA can be obtained from dermatome strips of human skin after snap-freezing, homogenization, and lyophilization. Advantages of this technique include the large yield, the ease, and the minimal manipulation of the skin; a significant disadvantage is that the dermatome sections contain some dermal cells. A purer sample of epidermal mRNA can be obtained when skin samples are incubated with the enzyme dispase and epidermal sheets are harvested and used as sources of RNA. RNA from the epidermal sheets appears to be intact. If epidermal sheets are subjected to trypsinization and then cell-sorted into CD1a positive and negative single-cell populations, lysates of these cells also can be used as sources of mRNA. As the purity of the cells used as the RNA source increases, the amount of manipulation required prior to RNA isolation increases, and the RNA yield decreases. However, even extremely small quantities of specific mRNA, prepared by these techniques, can be detected by reverse transcription and PCR amplification. Depending on the questions being asked, one of these three techniques of mRNA isolation may prove suitable for investigators studying the epidermis.

Introduction of an exogenous gene into a cell, or transfection, is now a popular technique for studying gene function and regulation and may prove a means of "repairing" genetic abnormalities. The laboratory of Dr. Miroslav Blumenberg at New York University has studied a number of methods by which DNA may be introduced into cultured keratinocytes. A method developed in Dr. Blumenberg's laboratory appears to be highly efficient: adsorption of the DNA to the cell surface in the presence of a polycation, known as polybrene, followed by uptake of the DNA by the cell, facilitated by an organic solvent, such as DMSO. One method not tested in this study, the use of a gene carried by a retroviral vector, was previously described by Garlick, Katz, Fenjves, and Taichman in the November 1991 issue of the *JID*. Proviral DNA, the product of reverse transcription of the retroviral RNA, is integrated into the DNA of the host cell. By this method, a single copy of the gene being carried by the retroviral vector is present in transduced cells. Gene transfer by this method appears to occur in stem cells, but the result is stable clones of the cells which carry the gene. This method, and the ones described by Dr. Blumenberg, should allow scientists to transfer genes to keratinocytes for study and possibly to use transplanted keratinocytes as carriers of missing genes.